A non-equilibrium dynamic mechanism for the allosteric effect

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(Dated: February 2, 2008)

Allosteric regulation is often viewed as thermodynamic in nature. However protein internal motions during an enzymatic reaction cycle can be slow hopping processes over numerous potential barriers. We propose that regulating molecules may function by modifying the nonequilibrium protein dynamics. The theory predicts that an enzyme under the new mechanism has different temperature dependence, waiting time distribution of the turnover cycle, and dynamic fluctuation patterns with and without effector. Experimental tests of the theory are proposed.

PACS numbers: Valid PACS appear here

A prominent property of enzymes (protein catalysts) is that their catalytic activities can be regulated. Enzymes that are allosteric have two or more binding sites. Effects of ligand (effector) binding or reaction on one site can propagate to another distant catalytic site and affect its activity. Understanding the allosteric mechanism(s) is an important topic in structural biology. Conventional models assume that effector binding modifies the equilibrium confomational distribution of allosteric proteins[1]. Recent proposed "dynamic models" emphasize entropic (or the accessible configurational space) changes due to effector-binding induced modification of protein fluctuation patterns [2, 3, 4, 5, 6]. Close examination reveals that these models actually share some basic ideas with the conventional models [7]. In summary allosteric regulation is generally believed to be "fundamentally thermodynamic in nature"[8]. For later discussions, we characterize these existing models as being driven by "thermodynamic regulation". However, here we argue that the above thermodynamic description may be incomplete, and propose an alternative "nonequilibrium dynamic regulation mechanism" . This idea is inspired by experimental and theoretical studies on dynamic disorder, the phenomena that the "rate constant" of a process is actually a statistical function of time due to slow protein conformational motions [9, 10, 11, 12, 13].

In this work we specifically examine allosteric regulation of enzymatic reactions. Furthermore, we consider the case of positive regulation (i.e., effector binding results in higher activity) unless specified otherwise. The catalytic site being regulated can be described by a few slow global conformational modes (here we assume one for simplicity) and local conformational changes involving atomic rearrangement (here refering as the reaction coordinates). For barrier crossing processes, a system spends most of the time near the potential minima, and the actual barrier-crossing time is transient. Therefore, one can reduce the potentials further to one-dimensional projections along the conformational coordinate, and approximate transitions along the reaction coordinate by rate processes between the one-dimensional potential curves. Similar description has been used in other contexts (e.g., protein motor studies [14]). Protein dynamics is affected by substrate binding. A minimal model representing the states of a catalytic site is: E (empty), S (substrate bound), P(product bound). Fig. 1a illustrates an example used in this work. The protein states are described by potential curves along the conformational coordinate with localized transitions between them. For an enzymatic cycle, a substrate molecule first binds onto the catalytic site (E→S), then forms a more compact complex from which a chemical reaction takes place (S→P), and finally the product is released (P→E). In the more familiar discrete kinetic form, the overall process can be represented as $E + S \rightleftharpoons ES \rightleftharpoons ES^* \rightleftharpoons EP \rightleftharpoons E + P$, with E, S and P refer to the enzyme, substrate, and product respectively. Notice that in general, the optimal conformational coordinates for reactant binding, the chemical reaction, and product release may not be the same (as also suggested experimentally [15]), and some conformational motion is necessary during the cycle. Dynamics of the reduced system can be described by a set of over-damped Langevin equations coupled to Markov transitions [16], $\zeta_i \frac{dx}{dt} = -\frac{dU_i(x)}{dx} + f_i(t)$, where x represents the conformational coordinate, $U_i(x)$ is the potential of mean force at a given substrate binding state, ζ_i is the drag coefficient, and f_i is the random fluctuation force with the property $< f_i(t) f_i(t') >= 2k_B T \zeta_i \delta(t - t')$, with k_B the Boltzmann's constant, T the temperature. Chemical transitions accompany motions along the conformational coordinate with x-dependent transition rates. For simplicity we leave the more general description, the generalized Langevin equation [13, 16, 17], for future studies. The dynamics can be equally described by a set of coupled Fokker-Planck equations (here we only consider the

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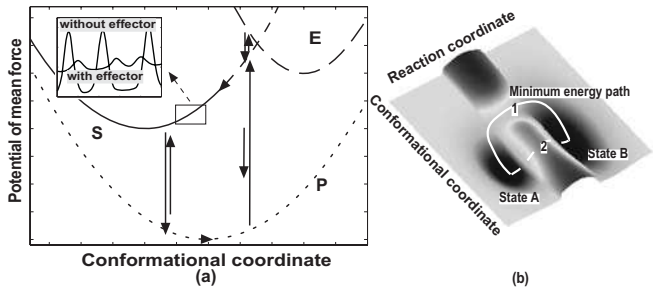

FIG. 1: A minimal model for the catalytic site (a)The free energy curves represent three distinct catalytic site binding states (E, S, P) projected onto the conformational coordinate x. The inlet illustrates that the smooth potentials are actually coarse-grained over rugged potential surfaces. Effector binding at a distant site may modify the roughness of the potentials . (b) Two classes of reaction pathways along a multi-dimensional surface. Path 1 is along the long but low barrier minimum energy path. Path 2 is a short high barrier corner-cutting path.

steady state),

$$-\frac{D_i}{k_BT}\cdot\frac{\partial}{\partial x}\left(-\frac{\partial U_i}{\partial x}\rho_i\right)+D_i\frac{\partial^2\rho_i}{\partial x^2}+\sum_{j\neq i}(k_{ij}\rho_j-k_{ji}\rho_i)=0 \quad (1)$$

Where $D_i = k_BT/\zeta_i$ the diffusion constant, k_{ij} the transition matrix element, and ρ_i the probability density to find the system at position x and state i. For simplicity we dropped the dependence of U_i and ρ_i on x in the above equation and later discussions. This is a unified framework for describing allosteric regulation. The existing models can be regarded as special cases with the conformational coordinate discretized [7]. The "thermodynamic regulation" models assume that effector binding at a remote site can affect the dynamics at the catalytic site by modifying U_i. While differing in details, these models assume a quasi-equilibrium distribution along the conformational coordinate, thus a thermodynamic treatment is appropriate [7]. However, protein conformational fluctuations can be very slow (e.g. from ms to minutes [2, 13, 15]), which is comparable or even slower than the enzyme turnover time. Consequently, protein fluctuations may not have fully accessed the conformational space, and thus not be in equilibrium. Variation of the dynamic properties along the conformational coordinate can have a dramatic effect on the apparent protein activity. Our recent theoretical analysis showed that the observed slow protein conformational dynamics can be explained by rugged protein potential surfaces[17]. During relative motions between two protein parts, numerous noncovalent bonds(residue pairs with electrostatic, hydrophobic/hydrophilic, steric interactions, etc.) may form and break with associated local conformational changes. These processes are in general uncorrelated with each other, which result in rugged potentials [18]. The relative motions are then characterized by hopping over numerous potential barriers (refer to inlet of Fig. 1a). For a potential curve with random ruggedness, Zwanzig showed that the barrier-hopping process can be approximated by diffusion along a coarse-grained smooth potential with an effective diffusion constant $D = D_0\exp(-(\epsilon/k_BT)^2)$, where D_0 is the bare diffusion constant, and ϵ is the potential roughness parameter [19]. The reported value of ϵ is 2-6 k_BT [20]. With $D_0 = 10^{-6}cm^2/s$, D can be reduced to $1\mathring{A}^2/s$ with $\epsilon \sim 4.8k_BT$. Thus internal diffusion can be a rate limiting step for enzymatic reactions and in principle can be regulated by allosteric effects (see Fig. 1). Further studies are necessary to clarify the atomic view of the proposed potential roughness regulation. The effective diffusion can be accelerated by inducing local conformational changes and synchronizing the breaking and formation of the noncovalent bonds. It may be also related to the coupling mechanism between global and local vibrational modes dicussed by Hawkins and McLeish [5].

In addition to being a theoretical possibility, the nonequilibrium regulation mechanism also has the following improvements over the conventional regulation mechanisms. First, it is a more effective way to regulate the enzyme activity than the thermodynamic regulation (conformational and entropic) mechanisms. To increase the activity by 10^{10} through an Arrhenius process, the activation "free energy" barrier needs to be lowered by 23 k_BT. Similar amount of free energy change is needed for an equilibrium population shift mechanism over the reactant conformational space. On the other hand, for an internal diffusion limited process, the reaction rate is linearly dependent on the effective diffusion constant. To increase the activity by the same 10^{10}, the lower bound of the roughness parameter only needs to be adjusted by $\sim 5k_BT$.

Secondly, compared to the conformational change mechanism, the nonequilibrium dynamic regulation mechanism has less requirements on the mechanical properties of the protein, similar to the proposed equilibrium dynamic models. The distance between the two binding sites of an allosteric protein can be far (e.g., 15 nm for the bacterial chemotaxis receptor [21]). Under the conformational change mechanism, effective coupling of the two sites requires a faithful finely tuned transmission of the mechanical strain due to ligand binding from one site to another one through a set of mechanical stress relaying network. These network residues must have mechanical properties distinctive from other residues to minimize energy dissipation to the surroundings. Otherwise, a significant portion of the effector binding energy would be wasted. In other words, coupling between these relaying residues and others should be minimized. By comparison, under the current nonequilibrium or the existing dynamics regulation mechanisms, the effect of effector binding can be highly nonlocal. Effector binding may affect the other site by finely regulating local structures far away from that site. By modifying the effective diffusion constant, these local modifications may affect the dynamics along the conformational coordinate x in our

TABLE I: 1 Model parameters. Here (E, S, P) refer to empty, substrate bound, and product bound, respectively. All energy units in this table are in reduced units, $k_BT = 1$. For simplicity,$D_E = D_S = D_P$. The prefactors of the rate constants are chosen so that the maximum values of k_{SE}, k_{PS}, and k_{ES} are approximately 10, 1.5, and 5, respectively.

	$S \leftarrow E$	$P \leftarrow E$	$E \leftarrow S$	$P \leftarrow S$	$E \leftarrow P$	$S \leftarrow P$
k_{ij}^0	2e2	2e-3	2e2	1.6e3	2e3	1.6e3
$U_{ij}^{0\dagger}$	3	3	3	6	3	6
L_{ij}	0.3	0.3	0.3	0.3	0.3	0.3
x_{ij}^e	0.65	0.65	0.65	-0.65	0.65	-0.65

formalism through coupling to global modes. The latters are composed of collective motions of residues within the catalytic site and those far from it. The effect manifests itself through larger root-mean-square deviation as observed in NMR, x-ray crystallography, and in molecular dynamics simulations [2, 6, 21]. The dynamics of the collective motions should be examined as well.

In our numerical calculations, the potentials are chosen to be harmonic potentials (see Fig. 1a), $U_i = \frac{1}{2}\kappa_i(x - x_{0i})^2 + U_i^0$, $\kappa_i = (1, 0.5, 0.4)$, $x_{0i} = (1, -0.5, 0)$, and $U_i^0 = (0, -1, -3)$. To model transitions between different states, we also model the transition state potentials by harmonic potentials, $U_{ij}^\dagger = \frac{1}{2}((x - x_{ij}^e)/L_{ij})^2 + U_{ij}^{0\dagger}$. The transition rate from state j to i is given by $k_{ij}(x) = k_{ij}^0 \exp[(U_j(x) - U_{ij}^\dagger(x))/k_BT]$ with parameters given in Table 1. By solving Eq. 1, the enzyme turnover rate (which measures how many substrate molecules can be transformed into product by an enzyme molecule per unit time) is calculated by $r = \int (k_{EP}\rho_P - k_{PE}\rho_E)dx$. Eq. 1 was solved with the algorithm developed by Wang *etal.* at given values of D and T [22]. The algorithm discretizes the conformational coordinate, and transforms the partial differential equations into a jump process over many discrete states with their normalized populations p (defined as the probability density integrated over the discrete regions) described in the form $\mathbf{Kp} = \mathbf{0}$. The composite $\mathbf{K}$ matrix contains transitions along both the conformational and reaction coordinates(see the original paper for details). Fig. 2a shows the calculated relative enzyme turnover rate as a function of the internal diffusion constant. While the diffusion constant is not a rate-limiting parameter at high D values (as compared to the chemical transition rates), at smaller values of D the turnover rate depends on the diffusion constant linearly which is a signature of the existence of diffusion-limited steps. Fig. 2b shows the temperature dependence of the turnover rate. With high values of D, the exponential $1/T$ dependence mainly comes from the Arrhenius dependence of the transition rates. However with small values of D, the turnover rate shows strong non-exponential dependence, since the effective diffusion constant D has a Gaussian dependence on $1/T$. Experiments can test the predicted different temperature dependence of enzyme activity with and without the effector provided it is regulated by the current mechanism. Fig. 2c also shows the waiting time distribution between two consecutive turnover cycles calculated using the formula derived by Gopich and Szabo [23],

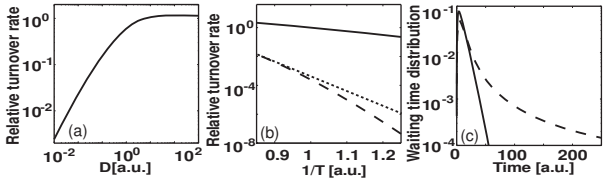

FIG. 2: Theoretical predictions. (a) Enzyme turnover rate as a function of the effective internal diffusion constant D. (b) Temperature dependence of the enzyme turnover rate. $D = D_0 \exp[-(\epsilon/k_BT)^2]$, where ϵ is the roughness parameter, and $D_0 = 10^3$. Solid line:$\epsilon = 0$. dashed line: $\epsilon = 4k_BT$, the dotted line gives the exponential dependence if the Arrhenius rate equation is assumed. The temperature dependence of D_0 is neglected in this calculation. (c) Turnover waiting time distribution with $D = 1$ (solid line) and $D = 0.001$ (dashed line).

$$P(\tau) = \mathbf{1}^\dagger \mathbf{V} \exp(\int_{\mathbf{0}}^{\tau} (\mathbf{K} - \mathbf{V})\mathbf{dt})\mathbf{Vp}/(\mathbf{1}^\dagger \mathbf{Vp}), \qquad (2)$$

where $1^\dagger$ refers to vector contraction, $\mathbf{K}$ the above composite transition matrix, $\mathbf{V}$ the transition matrix with only product release transitions as the sole nonzero elements, $\mathbf{p}$ the obtained steady-state population distribution. A system with low D values shows non-exponential distribution due to dynamic disorder. At high D values, effects of the dynamic disorder diminish and the distribution is exponential. Therefore, we predict that an enzyme functioning under the new dynamic regulation mechanism shows larger dynamic disorder effects. This can be tested by measuring consecutive single enzyme turnover time distributions with and without the effector, an extension of the work done by the Xie group [12].

For an enzymatic reaction under allosteric regulation, effector binding changes its reaction rate from k_1 to k_2. Let's define an effective free energy barrier change $\Delta\Delta G^\dagger = k_BT|\ln(k_2/k_1)|$, which must be due to the effector binding energy. Is it necessary that the effector binding energy be no smaller than $\Delta\Delta G^\dagger$? In a related question, for a system described by a multi-dimensional potential surface shown in Fig. 1b, is it possible that path 1 is dynamically comparable or even unfavorable than path 2? The answer to the latter is yes, provided that path 1 involves slow diffusion processes so that the average time for the transition along path 1 is even longer than path 2. A similar situation is discussed for tunneling pathways (e.g., proton-transfer reactions): the so-called corner-cutting large curvature tunneling and small curvature tunneling [24]. In this work we discussed that slow diffusion within a protein is physically possible due to the rugged potential surfaces. In his barrier crossing theory, Kramers derived dependence of the barrier

crossing rate on the barrier height, the drag coefficient, and other potential parameters of the system [25]. While most enzymatic reaction studies focus on barrier height changes, modification of the drag coefficient can affect enzyme activity (see also [26]). For proteins with rugged potentials, here we propose that the effective drag coefficient and thus protein activity can be tuned over a broad range by modifying potential roughness. In this case, the effector binding energy need not to be less than $\Delta\Delta G^{\dagger}$. The dynamic mechanism discussed in this work is related to other studies discussing protein dynamic properties and allosteric regulation by considering the effect of rugged potential landscapes [4]). However, there is also a fundamental difference. The current model treats the enzymatic reaction as a nonequilibrium problem in general. In their NMR studies of dihydrofolate reductase catalysis, Boehr et al. shows that the internal conformational motion is the rate-limiting step [15]. This system may provide a nice test system for the proposed mechanism. In addition, slow conformational dynamics has been observed for allosteric proteins [2] further supporting the validity of our formalism. Some experimental observations supporting the existing dynamic models are also consistent with the current model. Kim et al. and Popovych et al. proposed that the allosteric signal is transmitted through dynamical rather than conformational changes [21, 27]. We expect that the allosteric mechanism of a given protein has contribution from both thermodynamic regulation (the conventional conformational change mechanism [1] and the newly proposed entropic effect [2, 3, 4, 5, 6]), and nonequilibrium dynamic regulation proposed in this work. Different proteins may differ on which effect is dominant.

Acknowledgments

I thank Professors George Oster (UC Berkeley), Sung-Hou Kim (UC Berkeley), Hong Qian (U Washington), and Qiang Cui (U Wisconsin), Drs. Daniel Barsky, Ken Kim, Michael Surh, Todd Suchek at LLNL, Tongye Shen (UCSD), and Mr. Wei Min (Harvard) for helpful comments. JX is supported by a Lawrence Livermore National Laboratory Directed Research and Development grant, and by a Chemistry, Material, and Life Sciences Directorate fellowship. This work was performed under the auspices of the U.S. Department of Energy by the University of California, Lawrence Livermore National Laboratory under Contract No. W-7405-Eng-48.

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